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CHOLESTEROL-LOWERING ACTIVITY OF LACTIC ACID BACTERIA PROBIOTIC STRAINS *IN VIVO*

Cholesterol-lowering activity of probiotic strains of lactic acid bacteria genera Lactobacillus and Bifidobacterium in the in vivo experiments on the model of experimental hypercholesterolemia in mice was studied. It is established that the prophylactic scheme of introduction of probiotic cultures is more effective than therapeutic one for the manifestation of cholesterase activity of probiotic cultures. The most effective were the cultures: L. acidophilus and B. bifidum, as well as the composition B. bifidum + B. longum. Cholesterol-lowering activity of the studied strains and their compositions in this experiment ranged between 40-78%. It is noted that cholesterase activity of other studied strains was not lower, and in some cases, higher than that of most of the drugs currently used in cholesterinosis.

K e y w o r d s: probiotic lactic acid bacteria, cholesterol-lowering activity, cholesteraze activity, cholesterol-assimilating strains, cholesterinosis.

In recent years, the number of reports on the ability of lactic acid bacteria to lower serum cholesterol levels is increasing in the scientific literature. The ability of certain strains of normal microflora to assimilate and deconjugate precipitate bile acids as well as to destroy, bind and assimilate cholesterol is the basis of their cholesterol-lowering effect (the ability to reduce cholesterol levels) [5; 4; 7-9].

High cholesterol levels in the serum as a whole and in the low-density lipoprotein is a major risk factor for coronary heart disease and atherosclerosis, and for cerebrovascular atherosclerosis, hypertension, cancer of different parts of the digestive tract and some other pathologic conditions [2]. Thus, the purpose of the study was to establish the cholesterol-lowering activity of the previously selected strains of probiotic *Lactobacillus* and *Bifidobacterium* genera in the *in vivo* experiments on the model of experimental hypercholesterolemia in mice.

Materials and Methods. Probiotic strains of genera *Lactobacillus* and *Bifidobacterium* isolated from associative culture in laboratory studies of fermented biological material: *Bifidobacterium bifidum* VK-1, *Bifidobacterium longum* VK-2, *Lactobacillus acidophilus* IMV B-7279, *Lactobacillus casei* IMV B-7280, *Lactobacillus bulgaricus* IMV B-7281 were used as subjects of the study.

The authors used in the experiments white mice weighing 16-18 and 18-20 g, male mice of the *Balb/c* aged 2.5 months and female mice *Balb/c* aged 3 months. Experimental hypercholesterolemia was simulated in mice by feeding the animals with high-calorie diet (Table 1) for a week. Crystalline cholesterol with chemical purity of > 99% (Sigma, USA) was added to the diet. This model allows raising the serum cholesterol levels in mice by 46.54 ± 2.1% at an average as compared with intact mice.

Two schemes of administration of the probiotic strains – the prophylactic and therapeutic ones were worked out in the study. According to the prophylactic scheme mice of the experimental group received per os 0.3 ml of freshly prepared suspensions of the freeze-dried probiotic cultures, their combinations in concentrations of $3x10^8$ cells/ml, and mixed fodder during 4 days. On the fifth day the mice received high-calorie diet and continued to receive probiotic cultures every day until the end of the diet (seven days). On the first, third and seventh day since the beginning of high-calorie diet the level of total serum cholesterol in animals was determined [1]. Cholesterol-lowering activity (cholesterase activity) was calculated by a decrease of concentration of serum cholesterol in mice which

received high-calorie diet and probiotic cultures or their combinations in comparison with the control group of mice, which received only high-calorie diet. Cholesterol-lowering activity was evaluated in per cents from the control group of mice.

The therapeutic scheme provided co-administration of high-calorie diet and probiotic cultures in the diet of mice in the same doses as in the prophylactic scheme. The blood samples were also taken from the animals to analyze the level of total cholesterol on the first, third and seventh day of the experiment, respectively.

Two control groups of mice were used: the first (control) group included the intact mice, which diet included only the standard feed, the second one (control + diet) included mice which diet included only high-calorie diet with no addition of probiotic cultures.

Results and Discussion. In the previous experiments it was shown that all the studied strains of lactic acid bacteria were probiotic with high resistance to aggressive conditions of the gastrointestinal tract [3].

The previous experiments have proved that the selected strains, as well as compositions based on them, have high cholesterol-lowering activity *in vitro* [11].

The cholesterase activity of bacteria genera *Lactobacillus* and *Bifidobacterium* was determined in the study in the experimental model of hypercholesterolemia in mice. The results are shown in the figures (Fig. 1-6).

The data presented in Fig. 1. show that on the first day of using the therapeutic scheme of introduction of probiotic cultures into mice weighing 18-20 g maximum of cholesterase activity was observed for *L. acidophilus* – 31.15 ± 1.4%, while the minimum for compositions of *L. acidophilus* + *L. casei* – 3.0 ± 0.1%, and *L. casei* + *L. bulgaricus* – 4.32 ± 0.2%. At the same time for other cultures cholesterase activity ranged to 8.44-16.07 %. On the 3rd day of observation maximum cholesterase activity remained 37.11 ± 1.6% for *L. acidophilus*, the minimum value was 17.84 ± 0.7% for *L. casei*. The maximum value of cholesterase activity on the 7th day of observation was shown by the

culture *L. casei* as 62.28 ± 2.5%, minimum – by the composition *L. acidophilus* + *L. casei* as 28.70 ± 1.2%, respectively. For other cultures cholesterase activity was almost the same and varied within 43.01 ± 1.7%.

Fig. 2 shows cholesteraze activity for mice weighing 16-18g under administration of probiotic bacteria according to the therapeutic scheme. The maximum values of cholesterase activity were shown by *L. acidophilus* a*hilus* + *L. casei* as 43.38 ± 1.5%, and by *B. bifidum* + *B. longum* as 64.78 ± 2.7% on the 1st, 3rd and 7th days, respectively. On the first day minimum values were 16.66 ± 0.7% for *L. casei* and 16.63 ± 0.6% for *L. casei* + *L. bulgaricus*, on the third and seventh day they were 35.19 ± 1.3 and 48.16 ± 1.8%, respectively for *L. casei* + *L. bulgaricus*. On the seventh day of observation the average value of cholesterase activity was 33.0 ± 2.3%, which is by 13.32 ± 0.5% more than for mice weighing 18-20g. The obtained data suggest that it is easier to restore the organisms of young mice under the therapeutic administration of probiotic cultures, than the organisms of more mature mice.

Fig. 3 and 4 demonstrated the cholesterase activity of probiotic cultures in male mice *Balb/c* aged 2.5 months in prophylactic and therapeutic schemes of probiotic cultures administration, respectively.

For prophylactic scheme of administration of probiotic cultures (Fig. 3) cholesterase activity for all strains and their compositions were characterized by practically the same values and amounted to 33.63 ± 1.4% on the 1st day, 45.41±1.6% on the 3rd day, and 65.29 ± 2.6% on the 7th day, respectively. The maximum value of cholesterase activity on the 7th day of observation was characteristic of the culture *L. acidophilus,* it was 69.58 ± 2.8%. *L. acidophilus* and culture composition of *B. bifidum* + *B. longum* were the most effective for administration by the prophylactic scheme. The same trend remained for the medical(therapeutic ?) scheme (Fig.4). On the first day of the experiment the average cholesteraze activity ranged from 8.68% for *L. casei,* to 19.87-20.98 % for *L. acidophilus, B. bifidum* + *B. longum, L. casei* + *L. bulgaricus*. The average value of cholesterase activity on the third day of the therapeutic scheme was 35.11

± 1.4%. By the 7th day cholesterase activity increased minimum to 56.5% for *L. casei* and *L. casei + L. bulgaricus*, maximum to 65.84 ± 2.6% for *B. bifidum + B. longum*.

Fig. 5 and 6 showed the data for cholesterase activity of female mice *Balb/c* aged 3 months when using the prophylactic and therapeutic schemes of administering the probiotic bacteria, respectively. In this case, the prophylactic scheme administration also showed higher values of cholesterase activity than the therapeutic one. For prophylactic scheme administration (Fig. 5) the cholesteraze activity amounted to 37.01 ± 1.4% on the 1st day, 57.11 ± 2.3% on the 3rd day, 68.37 ± 3.0% on the 7th day. The maximum of cholesterase activity on the 7th day of observation was shown by the culture *L. acidophilus* as 78.04 ± 3.0% and by the composition *B. bifidum + B. longum* as 74.08 ± 3.0%.

The therapeutic scheme (Fig. 6) was characterized by slightly lower average values of cholesterase activity: 26.63 ± 1.1 % for the 1st day, 38.57 ± 1.5% for the third day, and 58.82 ± 2.4 % for the 7th day. The maximum value of activity was found for the culture *L. acidophilus* as 69.59 ± 2.8%, for other cultures it varied between 51.75 – 60.03 % on the 7th day of observation.

Recent researches in this field conformed completely to these experimental data [10].

As can be seen from all figures, regardless of breed, age, sex, body weight of mice and administration scheme of probiotic cultures their cholesterase activity increases to the seventh days of observation. It should also be noted that the prophylactic schemes of administration of probiotic cultures had higher values of cholesterase of bacteria than therapeutic ones. This suggests that the prevention of disease is the best treatment.

L. acidophilus and *B. bifidum*, as well as composition *B. bifidum + B. longum* were the most effective cultures used for treatment of mice with hypercholesterolemia. At the same time, it should be noted that the cholesterase activity of the other studied strains was not lower, and in some cases even higher

than that of most of the drugs currently used in cholesterinosis, for example Lovastatin, Fluvastatin, Atorvastatin and others [6].

Cholesterol-lowering activity of the studied strains and their compositions in the experiment ranged between 40-78%. In the future it is planned to increase the percentage of cholesterase activity by more detailed working out of administration schemes and doses of cultures, as well as the selection of combinations and ratios of strains in these combinations.

Thus, the selected cultures of lactic acid bacteria could potentially be used to create on their basis new probiotics to reduce serum cholesterol in humans. Probiotics that contain cholesterol-assimilating strains of lactic acid bacteria can efficiently complete the complex therapy of patients with cardiovascular, cancer and other diseases.

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ГИПОХОЛЕСТЕРИНЕМИЧЕСКАЯ АКТИВНОСТЬ ПРОБИОТИЧЕСКИХ ШТАММОВ МОЛОЧНОКИСЛЫХ БАКТЕРИЙ *IN VIVO*

Резюме

Изучена гипохолестеринемическая активность пробиотических штаммов молочнокислых бактерий родов Lactobacillus и Bifidobacterium в опытах in vivo на модели экспериментальной гиперхолестеринемии у мышей. Установлено, что профилактиечская схема введения пробиотических культур является более эффективной, чем лечебная, для проявления холестеразной активности культурами. Наиболее эффективными оказались культуры: L. acidophilus и B. bifidum, а также композиция B. bifidum + B.

longum. Отмечено, что холестеразная активность остальных изученных штаммов была не ниже, а в некоторых случаях и выше, чем большинства лекарственных препаратов применяемых в настоящее время при холестеринозе.

Ключевые слова: пробиотик, молочнокислые бактерии, гипохолестеринемическая активность, холестерин-ассимилирующие штаммы, холестериноз.

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ГІПОХОЛЕСТЕРИНЕМІЧНА АКТИВНІСТЬ ПРОБІОТИЧНИХ ШТАМІВ МОЛОЧНОКИСЛИХ БАКТЕРІЙ *IN VIVO*

Резюме

Вивчена гіпохолестеринемічна активність пробіотичних штамів молочнокислих бактерій родів Lactobacillus та Bifidobacterium в дослідах in vivo на моделі експериментальної гіперхолестеринемії у мишей. Встановлено, що профілактична схема введення пробіотичних культур є більш ефективною, ніж лікувальна, для прояву холестеразної активності культурами. Найбільш ефективними виявилися культури: L. acidophilus та B. bifidum, а також композиція B. bifidum + B. longum. Відмічено, що холестеразна активність інших вивчених штамів була не нижче, а в деяких

випадках і вище, ніж у більшості лікарських препаратів, що застосовуються в теперішній час для лікування холестеринозу.

Ключові слова: пробіотик, молочнокислі бактерії, гіпохолестеринемічна активність, холестерин-асимілюючі штами, холестериноз.

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Table 1

Composition of diet based on corn meal to feed mice

Components	Quantity, g
Corn meal	153.5
Butter	27.2
Wheat bran	72.6
Soybean meal	98.0
Kitchen salt	0.9
$CaHPO_4$	5.4
$CaCO_3$	3.3
Vitamins, macro- and microelements*	1.5

Comments: The composition of diet consists of the following vitamins, macro- and microelements: riboflavin – 1.76 μg; pantothenic acid – 8.80 μg; niacin – 8.80 μg; vitamin B_{12} – 8.80 μg; choline chloride – 176.00 μg; vitamin A – 1760 lU; vitamin D_3 – 176 lU; vitamin E – 4.4 lU; and also complex of macro- and microelements: selenium – 39.6 μg, iodine – 300 μg; iron – 19.8 μg; manganese – 11 μg, copper – 2.2 μg, zinc – 39.6 μg per 1 kg of feed.

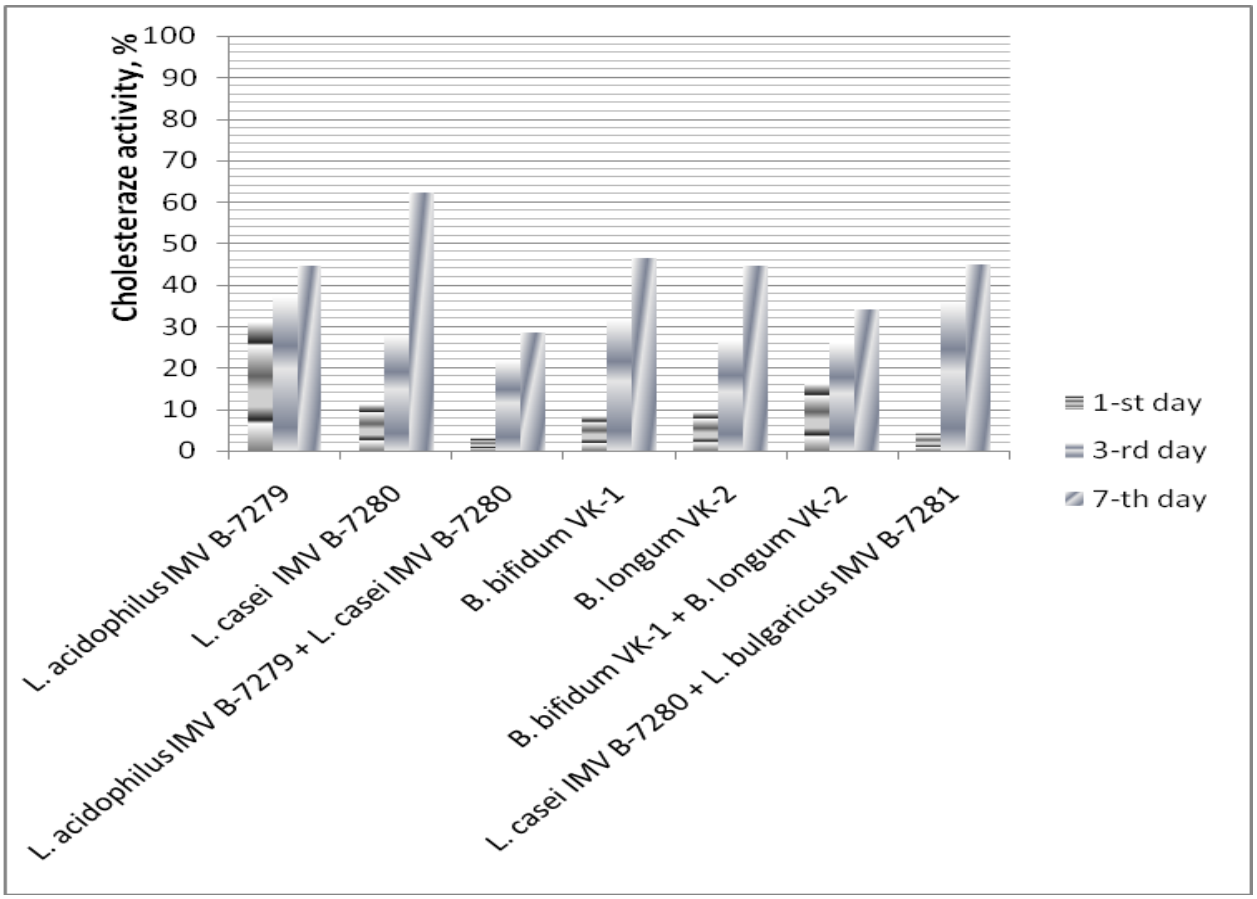

Fig.1. Dependence of cholesteraze activity of lactic acid bacteria and their compositions on observation time under therapeutic scheme administration of probiotic cultures ($P < 0.05$).

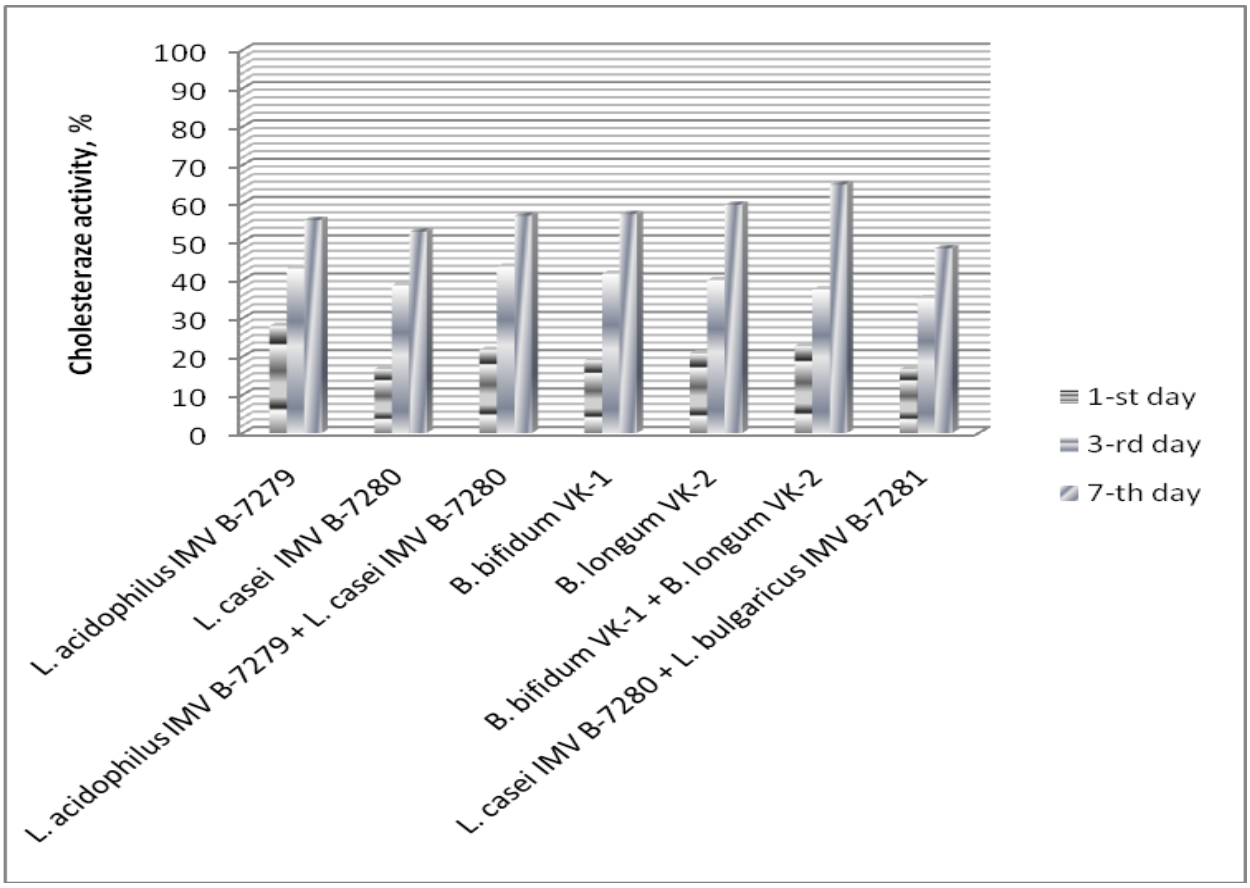

Fig.2. Dependence of cholesteraze activity of lactic acid bacteria and their compositions on observation time for mice weighing 16-18 g under the therapeutic scheme administration of probiotic cultures ($P < 0.05$).

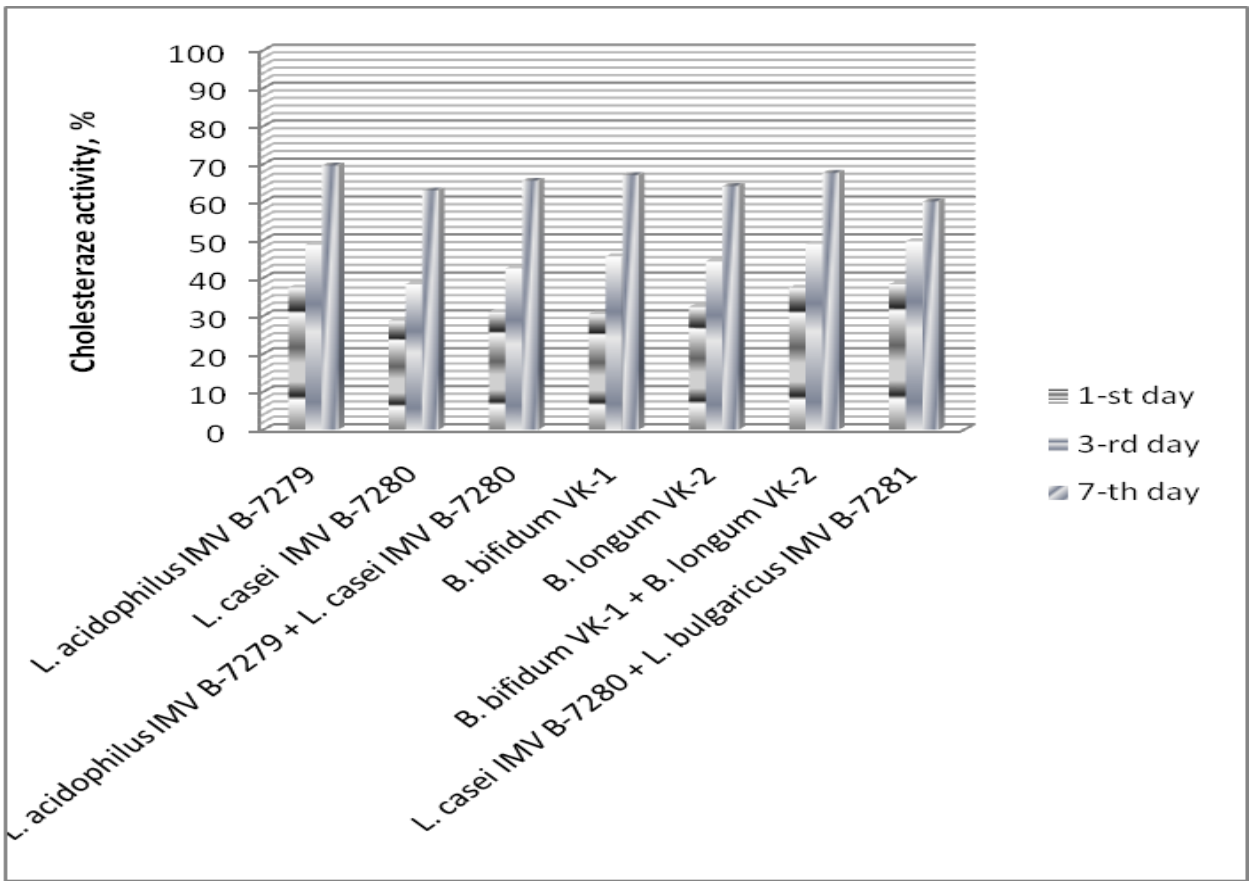

Fig.3. Dependence of cholesterase activity of lactic acid bacteria and their compositions on the days of observation for male mice *Balb/c* aged 2.5 months when using the prophylactic scheme administration of probiotic cultures ($P < 0.05$).

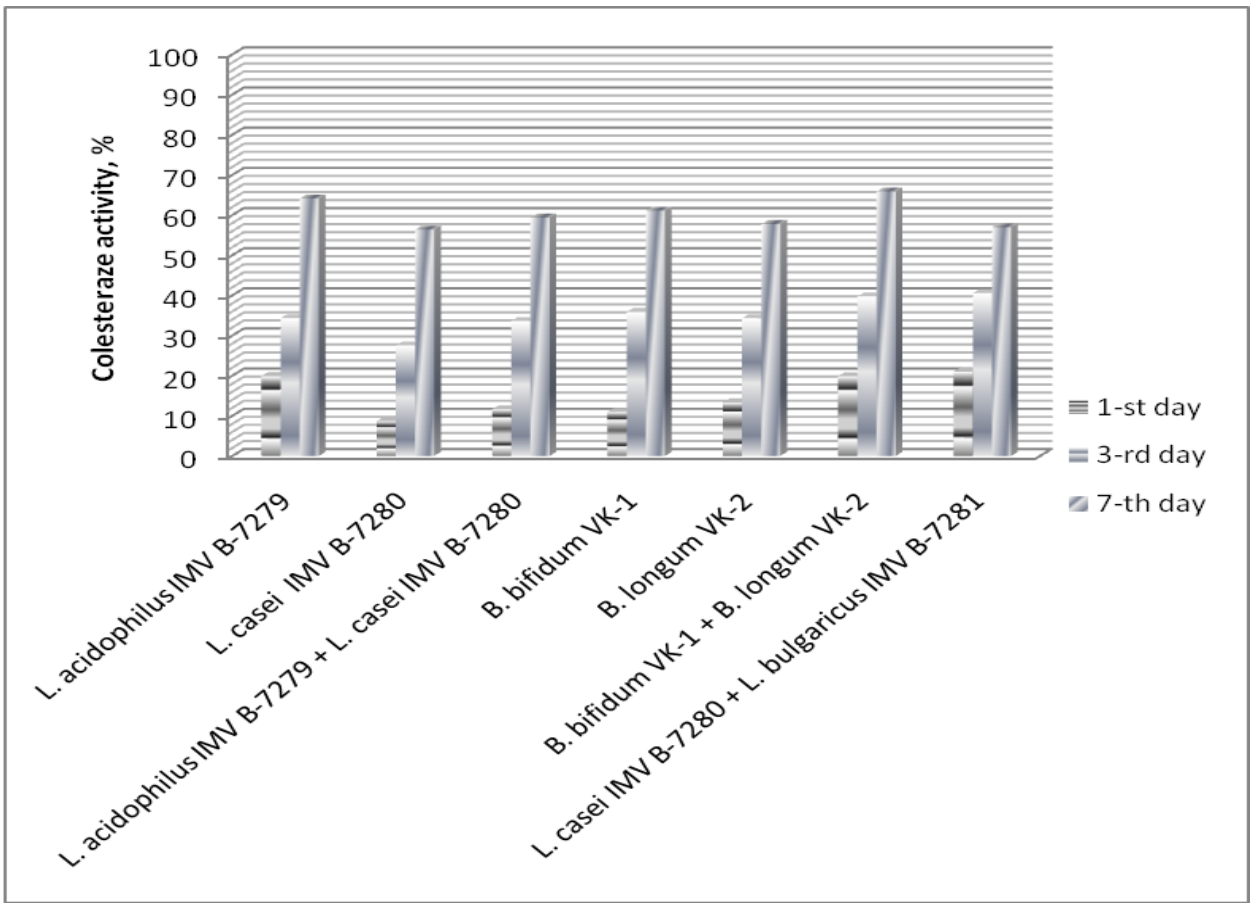

Fig.4. Dependence of cholesterase activity of lactic acid bacteria and their compositions on the days of observation for male mice *Balb/c* aged 2.5 months when using the therapeutic scheme administration of probiotic cultures ($P < 0.05$).

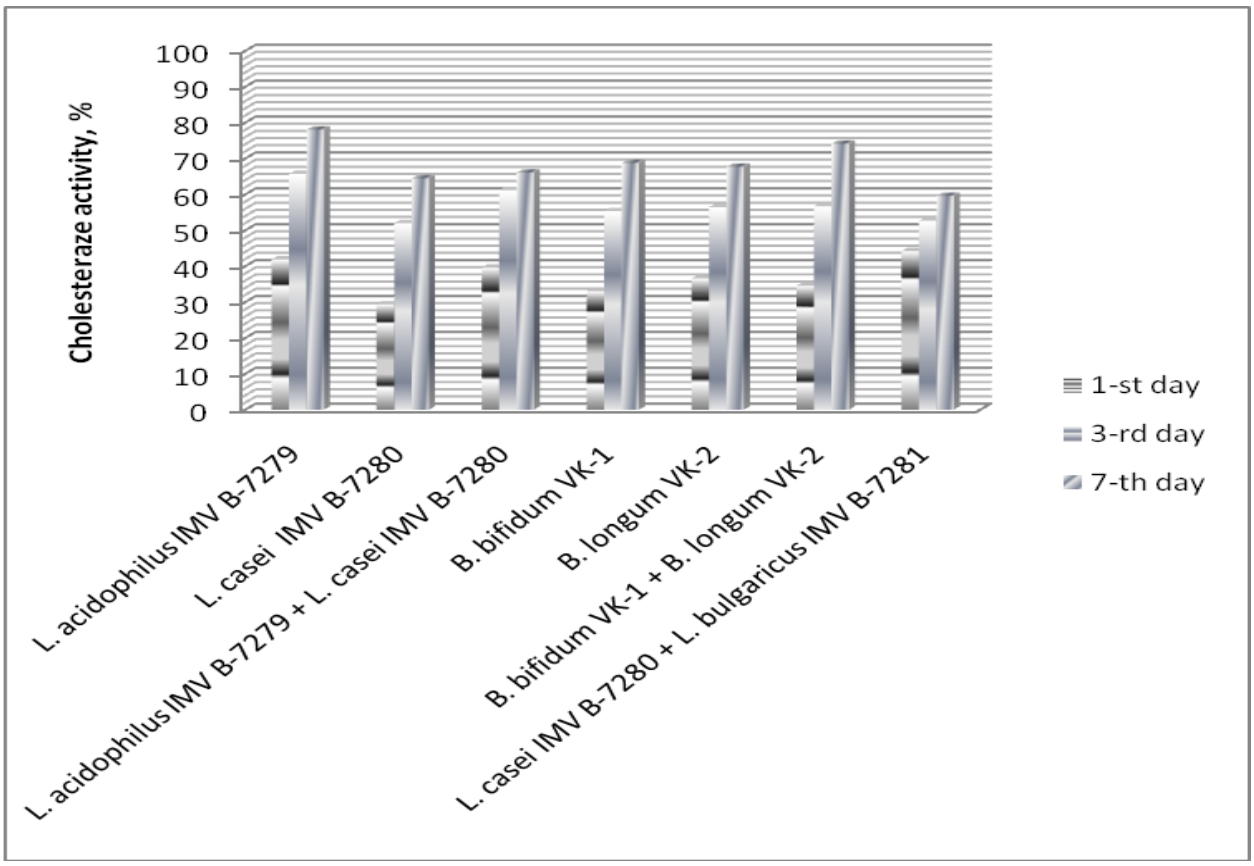

Fig.5. Dependence of cholesteraze activity of lactic acid bacteria and their compositions on the days of observation for female mice *Balb/c* aged 3 months when using the prophylactic scheme administration of probiotic cultures ($P < 0.05$).

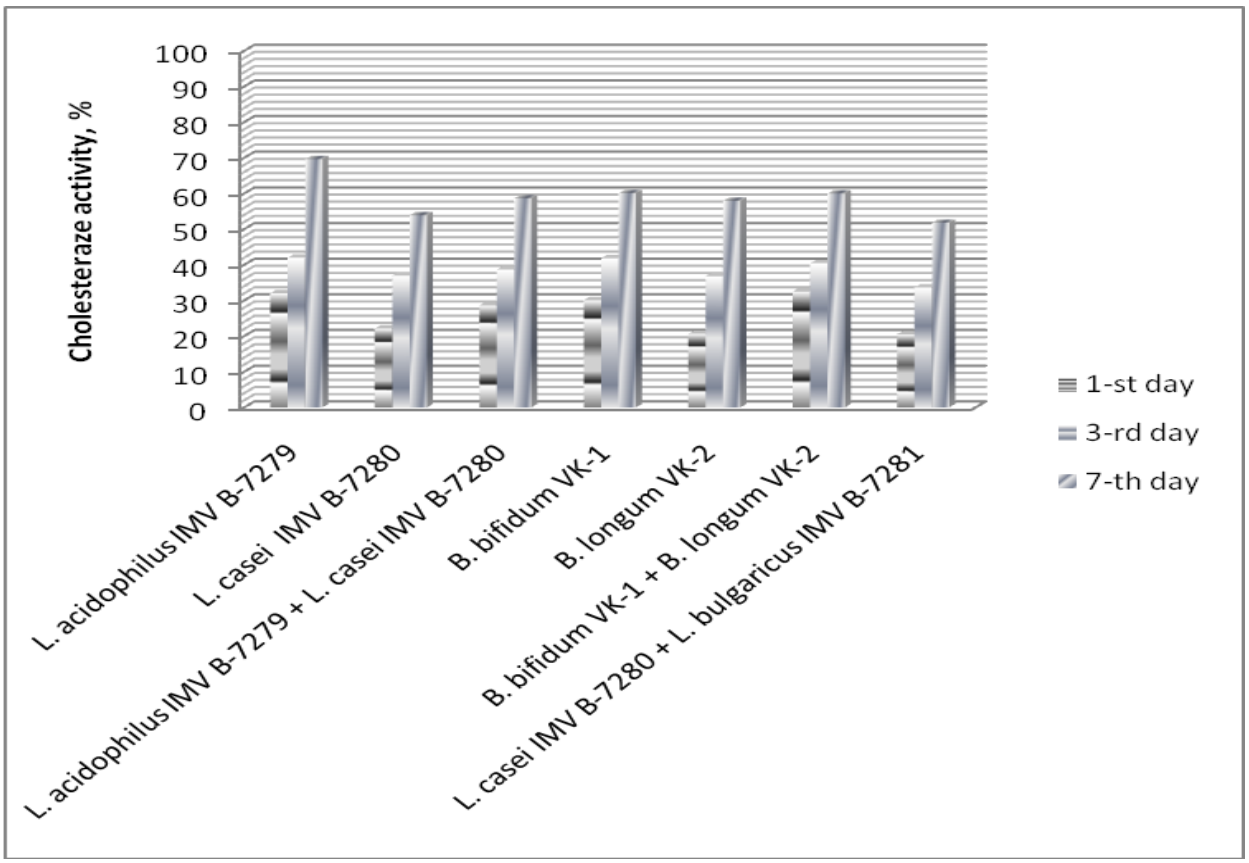

Fig.6. Dependence of cholesteraze activity of lactic acid bacteria and their compositions on the days of observation for female mice *Balb/c* aged 3 months when using the therapeutic scheme administration of probiotic cultures ($P < 0.05$).